

EXHIBIT B

Priming of both B cell anti-HIV envelope responses by vaccination is essential for the long lasting containment of viral replication in macaques infected with an R5-tropic SHIV isolate.

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ABSTRACT

Vaccines eliciting cellular anti-HIV/SIV responses but no neutralizing antibodies may more effectively control infection of primates by X4- or X4R5-tropic viruses than R5-tropic viruses, which are preferentially transmitted in humans. To determine whether long lasting control of R5-tropic virus replication is achieved when the vaccine elicits neutralizing antibodies, we immunized macaques with the HIV envelope using the 'DNA-prime / protein-boost' methodology and depleted them of their CD8+ cells prior to challenge with the R5-tropic, SHIV_{SF162P4} virus. Although the presence in the vaccinated animals of neutralizing antibodies at the time of challenge significantly reduced plasma viremia during acute-infection, the extent to which viral replication was controlled during the chronic phase of infection was dependent on the rapidity with which potent anamnestic neutralizing antibody responses were developed following challenge. Thus, even though the control animals developed anti-viral responses they did so more slowly than the vaccinated animals, and as a result the former, but not the latter, animals remained viremic, with decreasing CD4+ T cell numbers during the period of observation and some died from AIDS. Our studies emphasize the importance of priming by vaccination the B cell responses against the HIV envelope to effectively control HIV-replication during chronic infection.

INTRODUCTION

Vaccination methodologies that elicit cellular anti-HIV/SIV responses in the absence of measurable neutralizing antibodies, offer partial protection to macaques challenged with the dual-tropic (R5X4) pathogenic SHIV89.6P virus ¹⁻³. The benefit offered by such vaccines is primarily evident during the chronic phase of infection, when a significant reduction in plasma viral load levels is recorded in the vaccinated, as compared to unvaccinated, animals. Importantly, most vaccinated animals are protected from the rapid depletion of their CD4+ T cells and the rapid progression to disease, hallmarks of SHIV89.6P-infection ⁴. However, the emergence of escape mutants has been recorded in some of these animals, which eventually developed AIDS ⁵. In addition, priming by vaccination of cellular anti-viral responses alone may not be an effective way to control infection by pathogenic R5-tropic viruses, such as SIVmac239 ⁶.

It is well established that passive administration of neutralizing antibodies to macaques prior to their exposure to virus (even to R5-tropic viruses) results in sterilizing immunity, but only when the neutralizing antibody titers at the time of viral exposure are high ⁷⁻¹⁷. Whether the various vaccination methodologies that are currently evaluated will be able to elicit and sustain for extended periods of time neutralizing antibodies at titers capable of offering sterilizing immunity, is unknown. It is also not well established whether vaccine-elicited neutralizing antibodies at titers that are suboptimal for sterilizing immunity, would offer any benefit to the vaccinee. Finally it is unknown whether the presence of memory B cells with anti-HIV envelope specificity will be beneficial to a vaccinated, but infected host.

To address the above issues, we immunized rhesus macaques with the HIV envelope derived from the R5-tropic SF162 and the related SF162ΔV2 viruses using the 'DNA-prime plus protein-boost' vaccination methodology. To reduce the impact of the cellular anti-HIV responses during

acute infection, and to primarily evaluate the protective role of antibodies during this period, we transiently depleted the CD8+ cells from these animals prior to their exposure to the R5-tropic, highly replication-competent SHIV_{SF162P4} virus^{18,19}. SHIV_{SF162P4}-infection of rhesus macaques is characterized by high plasma viral load levels (10^7 - 10^8 viral RNA copies per ml blood) during the acute phase of infection; varying levels of plasma viremia during the chronic phase of infection; a gradual depletion of CD4+ T lymphocytes; and a gradual progression to disease. We report that although the titers of neutralizing antibodies elicited during vaccination were not sufficient to offer sterilizing immunity, they significantly reduced the viral load during the acute phase of infection. In addition, shortly after exposure to virus the vaccinated animals rapidly developed high titers of *de novo* neutralizing antibody responses, which in combination with CTL responses effectively controlled viral replication during the chronic phase of infection. The control animals were slower in developing anti-viral responses, but such responses were present in these animals during chronic infection. Despite their ability to mount anti-viral responses, the control animals were not capable of effectively controlling viral replication and some succumbed to AIDS.

Our studies underscore the crucial role that neutralizing antibodies play in controlling replication of R5-tropic SHIVs both during the acute and chronic phases of infection. They strongly suggest that vaccines against HIV should not only prime for cellular responses against multiple viral antigens, but also for anti-HIV envelope B cell responses in order to limit viral replication during the early stages of infection and allow the development of *de novo* antiviral responses capable of controlling infection for extended periods of time.

RESULTS

Pilot vaccination studies

We previously reported that two rhesus macaques (H445 and J408) immunized with the gp140 form of the HIV envelope derived from the SF162 Δ V2 virus¹⁸ (Δ V2gp140), using the 'DNA-prime plus protein-boost' immunization methodology, developed strong neutralizing antibodies that, in the absence of CD8+ cells, reduced plasma viral load levels during acute infection by the highly-replication competent, R5-using SHIV_{SF162P4} virus¹⁸. Here we report that both animals remained negative for plasma viral RNA for over two years (Fig. 1a), with stable numbers of CD4+ and CD8+ T lymphocytes (Fig. 1b and 1c, respectively), no signs of disease and no evidence for the emergence of escape viruses. During this period, the anti-HIV envelope antibody titers remained stable (Fig. 1d). CTL anti-viral responses against the SIV gag (primarily) and the HIV env proteins were measurable during chronic infection in these animals (Fig. 1e).

To evaluate in more detail the role of CTLs and neutralizing antibodies in controlling viral replication during chronic infection, 886 days post-challenge we administered the human/mouse hybrid anti-CD8 MAb cm T807^{20,21} in these animals. The CD8+ cell numbers were thus artificially depleted for 7 days in animals H445 and 14 days in animal J408 (Fig 1 c). The observed duration of CD8+ cell-depletion is shorter than what is usually achieved with this anti-CD8 MAb^{20,21}. We believe that this is due to the fact that these two animals had been already infused with the mouse antiCD8 MAb, OKT8F,¹⁸ and thus had been sensitized against mouse IgG. Concomitant with this reduction in CD8+ cell numbers, a reduction in cellular-mediated activity (Fig. 1e) and a burst in viremia (Fig.1a) (13,084 and 115,475 RNA copies per ml in animal H445 and J408, respectively) was recorded in the periphery. The fact that weak SIV gag-specific cellular responses were recorded in PBMC collected at the time of CD8+ cell-depletion (for example at 3 days post

cm T807 administration), suggests the presence of CD4+ cells with anti-viral activity in these animals. Once the CD8+ T cells re-appeared in the periphery, an increase in cellular-mediated anti-viral activity was evident and a concomitant reduction in plasma viremia to undetectable levels was once again recorded. In parallel to the increase in plasma viremia upon CD8+ cell-depletion, a rapid increase in the anti-HIV envelope antibody titers was recorded in both animals (Fig 1d). These observations suggest that both the cellular and humoral arms of the immune system are functional in these two animals and that both types of immune responses most likely contribute in the effective long-term control of viral replication.

In contrast, the two control animals of this group (AT54 and A141) had sustained viremia throughout the entire period of observation. One of them (animal AT54) had very high levels plasma viral load during and following acute infection; decreasing numbers of CD4+ T lymphocytes; and died from SAIDS within 16 weeks post-challenge (Fig 1a-d)¹⁸. The second control animal (A141) had high levels of plasma viremia during acute infection and remained viremic during chronic infection (Fig 1a), with stable CD4+ and CD8+ T cell numbers in the blood for the first 520 days following infection (Fig 1b and c, respectively). Subsequently, both CD4+ and CD8+ T cell numbers decreased. During the entire period of observation the anti-HIV envelope binding antibody titers gradually increased (Fig 1d). This animal was euthanized at day 670 post-infection because of health complications unrelated to SHIV_{SF162P4}-infection. However, at necropsy this animal had appreciable viremia in the spleen (143,000 RNA copies per mg), inguinal (38,450 RNA copies per mg), auxillary (16,000 RNA copies per mg) and mesenteric (722 RNA copies per mg) lymph nodes. While CD8+ T lymphocytes were present in these tissues at necropsy, CD4+ T lymphocytes were undetectable (data not shown).

Immunization of a second group of animals

To confirm the above observations and to examine in more detail the protective role of vaccine-elicited neutralizing antibodies during the acute phase of infection, a second group of animals (K863, P655, I708 and N473) was similarly immunized with the envelope protein derived from the SF162 Δ V2 (as the first group of animals) or the related SF162 viruses²² (SF162gp140 and Δ V2gp140, respectively). Potent binding-antibody responses were generated in all four animals following the 'booster' immunization with the CHO-produced soluble oligomeric gp140 envelope proteins (Fig. 2a), similar to what we recorded with the first group of animals¹⁸. However, the titer of binding antibodies did not always correlate with the titer of neutralizing antibodies against the challenge virus, SHIV_{SF162P4} (Fig. 2b), as we previously reported for the first group¹⁸. In fact, out of the six immunized animals in these two groups, only two (J408, from the first group, and N473, from the second group) developed neutralizing antibody responses, potent enough to neutralize the challenge SHIV_{SF162P4} virus by 90% at a serum dilution of 1:5, during *in vitro* neutralization using activated human PBMC as target cells (Fig. 2b and¹⁸). At the end of the immunization protocol, weak anti-HIV envelope cellular mediated responses were recorded in three out of the four vaccinated animals (Fig. 2c).

Depletion of CD8+ cells prior to viral-challenge: Despite our inability to generate stronger anti-viral cellular mediated responses, we decided to deplete the CD8+ cells from these animals prior to their challenge with virus to eliminate any potential involvement of these cells during the early stages of infection and to better appreciate the benefits offered by the vaccine-elicited neutralizing antibodies during this period of time. CD8+ cell-depletion was achieved as previously reported for the vaccinated animals of the first group¹⁸, by administrating the anti-CD8 MAb

OKT8F (Fig. 3). An isotype control MAb, OKT3, was administered to the control animals M844 and C640. OKT8F-administration resulted in the transient depletion of CD8+ cells from the periphery, which lasted between 9 (animal P655, Fig. 3*d*) and 14 days (animal K863, Fig. 3*b*). During the same period, the CD8+ cells were also depleted from the axillary and inguinal lymph nodes examined (data not shown). Cellular-mediated anti-HIV envelope (or SIV gag) responses were not detectable during this period in the periphery (see below). Subsequently, CD8+ T lymphocytes gradually re-emerged in the periphery and the lymph nodes of the vaccinated animals (Fig. 3 and data not shown).

Inverse correlation between acute plasma viremia levels and titers of vaccine-elicited neutralizing antibodies: Both the vaccinated and the control animals of the second group became infected upon their intravenous exposure to SHIV_{SF162P4} (Fig. 4*a*). Peak levels of plasma viremia were recorded approximately 12-15 days post-challenge in all animals. Although the titers of vaccine-elicited neutralizing antibodies were not sufficient to offer sterilizing immunity, the differences in the peak viral load levels during acute infection between the vaccinated (Mean peak viral load of 1,794,000 and standard deviation of 891,900) and control animals (Mean peak viral load of 9,784,000 and standard deviation of 178,400) is statistically significant ($P: 0.004$, $R^2: 0.8985$) (Fig. 4*b*). Importantly, animal N473 with the strongest neutralizing antibody responses at the day of challenge (Fig 2*b*) had the lowest peak viremia levels during acute infection (7,180 RNA copies per ml). These observations are in agreement with those made with the first group of animals, where animal J408 with the highest neutralizing antibody titers at the time of challenge, had lower plasma viremia following infection (8,030 RNA copies per ml) than the second immunized animal, H445, which had weaker neutralizing antibody responses (Fig 1*a*) and ¹⁸.

Development of anamnestic anti-viral responses and effective long-term control of infection

All the vaccinated animals developed potent anamnestic binding anti-HIV envelope antibody responses during the first two weeks post-challenge (Fig. 4c). The vaccinated animals with the lower neutralizing antibody titers at the day of challenge (animals I708, K863 and P655) developed stronger anamnestic binding-antibody responses than the vaccinated animal with the strongest neutralizing antibody responses at the day of challenge (animal N473). In parallel, the titers of neutralizing antibodies increased in all vaccinated animals (Fig. 4d). The emergence in the vaccinated animals of strong neutralizing antibody responses coincided with the recorded decrease in plasma viremia during acute infection. All the vaccinated animals were able to effectively control viral replication during the chronic phase of infection (Fig. 4a), so that with the exception of animal P655, virus has remained undetectable in the periphery of these animals for almost two years following infection.

In the control animals, the appearance of binding and neutralizing antibodies (Fig. 1c,d) was delayed by one to two weeks as compared to the vaccinated animals and occurred following the initial reduction in plasma viremia during acute infection (Fig 1a). This reduction is therefore most likely due to the emergence of cellular-mediated anti-viral responses (Fig. 5). At the earlier time point tested (day 6 post-infection) the anti-viral cellular responses were undetectable, both in the vaccinated (which at the time lacked CD8+ cells) and the control animals (Fig. 5). CTL responses were, however, recorded in all animals at later time points. No difference in the rate at which cellular anti-viral responses were generated in the vaccinated and control animals was evident. During the period of observation, these responses were maintained in some, but not all animals.

The anti-SIV gag responses (Fig. 5a) were more potent than the anti-HIV env responses (Fig. 5b) in all animals and at all time points tested. PBMC from all animals were susceptible to PHA-mediated stimulation throughout course of infection (Fig. 5c). The control animal C640 with the highest set-point plasma viral load (Fig. 4a) had the strongest cellular anti-viral responses, in accordance with recent human data during chronic HIV-infection²³. However, despite the fact that this animal developed both neutralizing antibodies and potent cellular-mediated anti-viral responses, it failed to eliminate the virus from the periphery (Fig 4a).

We have monitored this second group of SHIV_{SF162P4}-infected animals for almost two years. All the vaccinated animals have stable CD4+ T cell numbers (Fig. 4e) and, with the exception of animal P655 (10^3 to 10^4 RNA copies per ml), they remain plasma RNA-negative. The control animal C640 remained plasma RNA positive (approximate 10^5 RNA copies per ml of plasma) for the duration of observations, with decreasing CD4+ T cells in the periphery (Fig. 4e) and eventually developed sAIDS. In contrast, the control animal M844 has remained plasma RNA negative and with stable CD4+ T lymphocyte numbers during the same period of observation, even though during acute infection this animal had similar high viremia as animal C640.

DISCUSSION

The ultimate goal of a vaccine against HIV is to prevent infection. To this end, an effective vaccine must elicit the generation of very high titers of neutralizing antibodies^{10,17} especially against R5-tropic viruses which are primarily transmitted in humans²⁴⁻³¹. Our challenge studies were conducted with the R5-tropic, highly replication-competent SHIV_{SF162P4} virus. This virus was chosen because in contrast to all the other known SHIVs, is R5-tropic **like the HIV isolates responsible for transmission in humans (refs)**. In addition, SHIV_{SF162P4}-infection of macaques

better mimics infection of humans by HIV, as compared to infection of macaques with highly pathogenic X4- or X4R5-tropic SHIVs, which induce an unusually rapid-depletion of CD4+ T lymphocytes and a very rapid progression to disease and to death^{4,32,33}. The envelope SHIV_{SF162P4} is closely related to the envelope immunogens SF162gp140 and SF162ΔV2gp140 immunogens used here. As a result we did not record differences between the SF162 and SF162ΔV2-immunized animals in their ability to control infection by SHIV_{SF162P4}. Future studies will establish which immunogen is more effective in eliciting antibodies capable of protecting from heterologous viral challenge. However, the current studies allowed us to establish the levels of neutralizing antibodies required to be elicited by the DNA-prime plus protein-boost vaccination methodology, in order to record a significant reduction in plasma viremia during infection upon heterologous viral challenge.

During our vaccination studies we were not able to elicit titers of neutralizing antibodies capable of offering sterilizing immunity to SHIV_{SF162P4}-challenge by the intravenous route. However, the vaccinated animals had significantly lower peak plasma viremia titers as compared to control animals, despite the fact that we depleted the CD8+ cells from the former and not from the latter animals prior to viral-exposure. The vaccinated animals with the strongest neutralizing antibody responses at the day of challenge had up to 4log₁₀ lower levels of peak plasma viremia as compare to the controls. Such a dramatic reduction in plasma viral load levels during acute infection is not usually observed in animals immunized with vaccines that elicit primarily (or exclusively) cellular-mediated, but not measurable neutralizing antibody, responses against the challenge virus^{1-3,6}. Therefore the significant reduction in viremia during acute infection recorded is primarily due to the neutralizing antibodies elicited during vaccination. However, it is also possible that vaccine-elicited antibodies may also have contributed to the elimination of viral

particles via non-neutralization-mediated mechanisms. Additionally, cellular responses that are not mediated by CD8+ cells could be elicited during DNA-immunization and participate in the early control of viral replication. Irrespective of the extent to which viral replication was reduced during acute infection, all vaccinated animals rapidly developed *de novo* binding and neutralizing antibodies. Such responses were also generated in the control animals but their emergence was delayed as compared to the vaccinated animals. In contrast, cellular anti-viral responses emerged at the same rate in the vaccinated and control animals. We assume that if the CD8+ cells were not artificially depleted from the vaccinated animals prior to viral challenge, cellular anti-viral responses would have emerged earlier in these animals.

Although most of the vaccinated animals remained aviremic during chronic infection and all have stable CD4+ T cells numbers, most control animals remain viremic and some had decreasing numbers of CD4+ T cell numbers in the periphery and eventually developed AIDS.

In a recent study, macaques immunized with a DNA-prime plus vaccinia-boost immunization regiment developed cellular, but not neutralizing antibody, responses against the challenge R5-tropic SIVmac239 virus ⁶. During acute infection, these animals had reduced viral load levels, as compared to controls, and developed robust cellular mediated antiviral responses, but not neutralizing antibodies. Despite the development of such responses, these animals progressed to disease as rapidly as the control animals. One of the differences between that study and ours is that in our case, neutralizing antibodies were present at the time of challenge and their titer rapidly increased following challenge. Thus, vaccination methodologies that prime both B and T cell responses maybe more effective in controlling infection by highly replication-competent R5-tropic viruses, that those primarily, or exclusively, priming the cellular arm of the immune system. Although our challenge studies were conducted with a virus that expresses an envelope that is

closely related to our immunogens, our studies suggest that vaccines the elicit even low titers of neutralizing antibodies against heterologous HIV isolates, could be very beneficial.

Overall our data indicate that vaccine-elicited suboptimal titers of neutralizing antibody against HIV will be able to limit viral replication during the early stages of infection, even in the absence of CD8+ cell-mediated anti-viral responses. The ability however of the immune system to control viral replication during the chronic phase of infection is not dependent on the extent to which viral replication is slowed down during acute infection, but on the rapidity with which *de novo* antibody and cellular antiviral responses are generated by the infected host. Thus, priming of B cell mediated anti-HIV responses by vaccination is crucial for the effective control of HIV infection.

METHODS

Vaccines and vaccination methodology: A detailed description of the vaccination methodology, as well as the generation and production of the various immunogens, was reported earlier³⁴. Briefly, the animals received three monthly immunizations with DNA vectors expressing the gp140 envelope form derived from the SF162 or SF162ΔV2 viruses²². 5-10 months later they were immunized a fourth time with DNA and at the same time with the corresponding recombinant gp140 proteins produced in CHO cells, purified as oligomers and adjuvanted in MF-59C (Chiron Co. Emeryville, CA). The control animal M844 received three administrations of the 'empty' DNA vector and the MF-59C adjuvant (mock-immunization).

Determination of CD3+, CD4+ and CD8+ T cell-numbers in the periphery and lymph nodes: Absolute CD3+, CD4+ and CD8+ T lymphocyte numbers were measured on a Coulter Epics XL.MCL Flow-cytometer using anti-CD3-FITC (clone SP34, BD, San Diego, CA), anti-

CD4-PE (clone M-T477, BD, San Diego, CA) and anti-CD8-PC5 (clone B9.11, Immunotech, Beckman Coulter). Appropriate anti-mouse Ig isotypes were used as negative controls.

Antibody-responses: a) Binding antibodies: Titers were determined throughout the immunization protocol and following viral-challenge as previously described ³⁴. Briefly, purified soluble oligomeric Δ V2gp140 and SF162gp140 proteins were adsorbed onto Immulon 2HB 96 well plates (ThermoLabsystems, Franklin, MA) by an overnight incubation at room temperature. Serially-diluted heat-inactivated (56°C for 35 minutes) sera collected from the immunized animals were added to the wells (0.1 ml per well) for one hour at 37°C . Pre-immunization sera were used as negative controls. Envelope-bound serum antibodies were detected with the use of goat anti-human IgG coupled to alkaline phosphatase antibodies (Zymed Immunochemicals, South San Francisco, CA) and the appropriate substrate/amplifier combination (Dako CO. Carpinteria, CA).

b) Neutralizing antibodies: Neutralization assays were performed using as target cells human PBMC that were activated for three days with PHA (Sigma, 3 $\mu\text{g}/\text{ml}$) as we previously described ³⁴. SHIV_{SF162P4} (100 TCID₅₀ in 50 μl of complete RPMI media containing 40 U/ml of IL-2 (Hoffmann-La Roche)) was pre-incubated in triplicate with an equal volume of serially diluted heat-inactivated pre-immunization, post-immunization, or post-SHIV-challenge sera for one hour at 37°C , in 96 well U-bottom plates (Corning). Pre-immunization sera served as controls for non-specific neutralization. To each well, 0.1 ml of complete media containing 0.4×10^6 PHA-activated human PBMC was added. Following a 24-hour incubation at 37°C , half the volume of each well was replaced with complete RPMI media. Following centrifugation of the plates (5 minutes at 2,000 rpm), half the volume of each well was again replaced with fresh media. The concentration of p27 antigen in the supernatant was determined with a commercially available kit (ZeptoMetrix Corporation, Buffalo, NY). The percent inhibition of infection at a given dilution of

sera collected post-vaccination or post-infection sera was calculated taking into account the non-specific neutralization recorded with sera collected prior to the initiation of immunization ³⁴.

CD8+ cell-depletion: a) **During acute infection:** The immunized animals were infused with the mouse anti-CD8 MAb OKT8F as previously described ³⁵. Control animals M844 and C640, were similarly treated with an unrelated MAb (OKT3). CD8-depletion was evaluated using the anti-CD8 MAb OKTA-FITC that recognizes a different epitope than that recognized by OKT8F (data not shown). b) **During chronic infection:** Depletion of CD8+ cells during the chronic phase of infection was achieved by infusing the anti-CD8 MAb, cm T807, as previously reported ²⁰.

SHIV_{SF162P4}-challenge: Seven weeks following the end of the immunization and one day following the last administration of the anti-CD8 antibody OKT8F, the animals were challenged by an intravenous administration of 100 TCID₅₀ of a cell-free stock of the SHIV_{SF162P4} virus ¹⁹.

Viral load measurements: The SIV bDNA assay (Bayer Reference Testing Laboratory, Emeryville, CA) was employed to quantify plasma SIV RNA copy numbers. The lower quantitation limit of this assay is 500 SIV RNA copies per ml.

ELISPOT: Cryopreserved PBMC were plated at a density of 2×10^5 per well onto wells of a 96-well flat-bottomed plate that were previously coated with anti-IFN- γ antibody (Ucytech # CT 126). Pooled peptides (15-mers with an 11 amino acid overlap) derived from the homologous SIVmac239 Gag or the related SHIV_{SF162P3} Env (NIAID, Reference and Reagent Program) were then added into the wells, at a final concentration of 1 μ g/ml for each individual peptide. As a positive control, Staphylococcus Enterotoxin B (SEB) was added to the wells at a final concentration of 5 μ g/ml. As negative control, PBMC were plated in medium alone. The cells were incubated for 24 hrs at 37°C and processed according to manufacturer's specifications using

the Monkey IFN- γ ELISPOT Kit (Ucytech). Only ELISPOT counts of twice the background were considered significant (over 20 counts per well).

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FIGURE LEGENDS

Fig. 1 Long-term suppression of SHIV_{SF162P4} replication in vaccinated macaques. Plasma viral load ✓

(a), plasma CD4+ T cell numbers (b), plasma CD8+ T cell numbers and (c) evolution of binding ✓ ✓

anti-HIV envelope antibody responses (*d*), in control (A141, ▼, and AT54, ◆) and vaccinated (J408, ◆, and H445, ■) animals following intravenous challenge with SHIV_{SF162P4}. Black arrows indicate the time of administration of the anti-CD8 MAb OKT8F prior to viral challenge and white arrows indicate the administration of the anti-CD8 MAb cm T807 during the chronic phase of infection. (†) Indicates the time of death (*e*). Anti-SHIV CTL responses during chronic infection were determined with the ELSIPOT assay using PBMC collected at the indicated time points following viral-challenge. Numbers in parenthesis indicate the time of PBMC-collection in respect to administration of the anti-CD8 MAb cm T807. IFN- γ production following stimulation with peptides derived from the HIV env (green) or the SIVgag (blue). Responses obtained with SEB (red) are also shown.

Fig. 2 Development of antibody responses during 'DNA-prime plus protein-boost' vaccination with the SF162gp140 and Δ V2gp140 proteins. *a*, Generation of binding antibodies against the soluble oligomeric gp140 HIV envelope proteins during three DNA administrations (black arrows) and following a fourth DNA administration in combination with the purified recombinant gp140 proteins (white arrow). Animals P655 (□) and N472 (○) were immunized with the SF162gp140 envelope immunogen and animals I708 (▼) and K863 (◆) with the related Δ V2gp140 envelope immunogen; *b*, neutralizing potency of serum antibodies at the day of viral-challenge against the challenge SHIV_{SF162P4} virus. Horizontal dotted lines indicate the 90, 70 and 50% levels of neutralization; *c*, cellular mediated responses at the end of the immunization schedule (2 weeks post protein-boosting).

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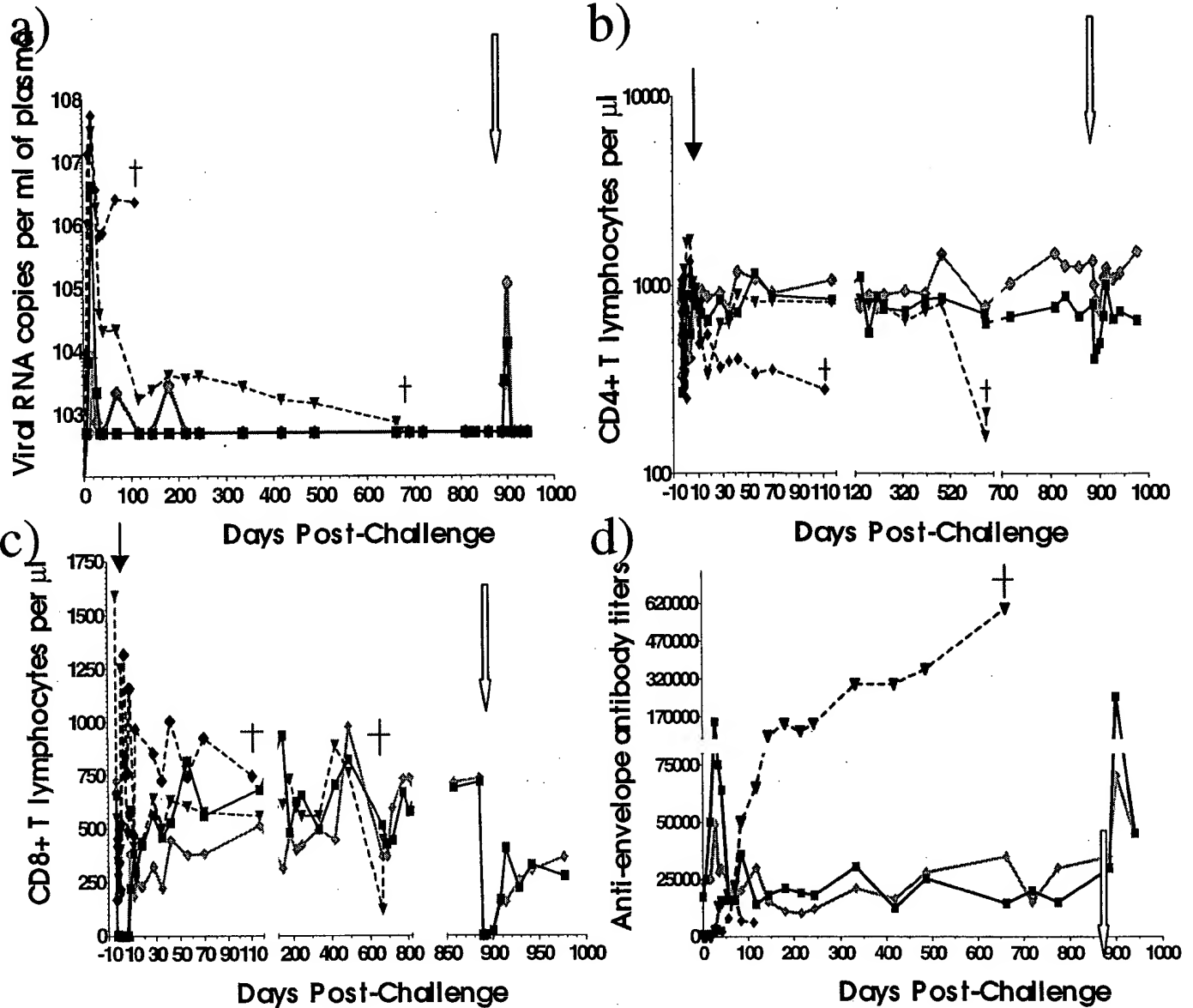
Fig. 3 Transient depletion of CD8+ cells from the vaccinated macaques. The vaccinated animals I708 (*a*), K863 (*b*), N472 (*c*) and P655 (*d*) were infused with the anti-CD8 MAb OKT8F, while the control animals M844 (*e*) and C640 (*f*) were infused with the isotype control MAb OKT3. Arrows indicate the time of OKT8F-infusion and the dotted line indicates the time of SHIV_{SF162P4}-challenge. ◇, CD8+ T lymphocytes; ●, CD4+ T lymphocytes; *, CD3+ T lymphocytes.

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Fig. 4 Control of SHIV_{SF162P4}-replication during acute and chronic infection. *a*, Plasma viral load levels; *b*, Inverse correlation between the peak plasma viral load levels during acute infection (red bars) and the anti-SHIV_{SF162P4} neutralizing potency of vaccine-elicited antibody responses at the day of viral-challenge (blue bars); *c* and *d*, Development of anti-HIV envelope binding (*c*) and anti SHIV_{SF162P4} neutralizing (*d*) antibody responses following infection; *e* CD4+ T cell numbers in the periphery following infection. Control animals: M844 x and C640,*. Vaccinated animals I708 ▼, K863 ◆, N472 ● and P655 ■. (†) Indicates the time of death.

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Fig. 5 Cellular anti-viral responses following immunization and during SHIV_{SF162P4} infection. *a*, Anti-SIVGag-directed responses; *b*, anti-HIV envelope-directed responses; and *c*, SEB-mediated responses were determined using the ELISPOT assay using cryopreserved PBMC collected two weeks following the end of the immunization protocol (Vaccination) and the indicated times following SHIV_{SF162P4} infection from the control (M844 ■ and C640 ■) and vaccinated (I708 ■, K863 ■, N472 ■ and P655 ■) animals.



e) Days Following SHIV_{SF162P4}-Infection

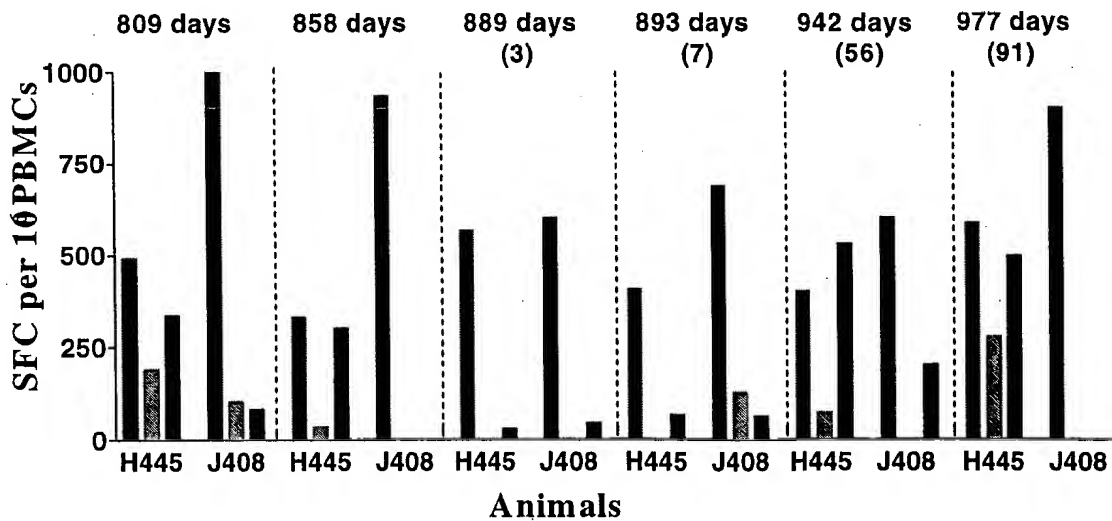
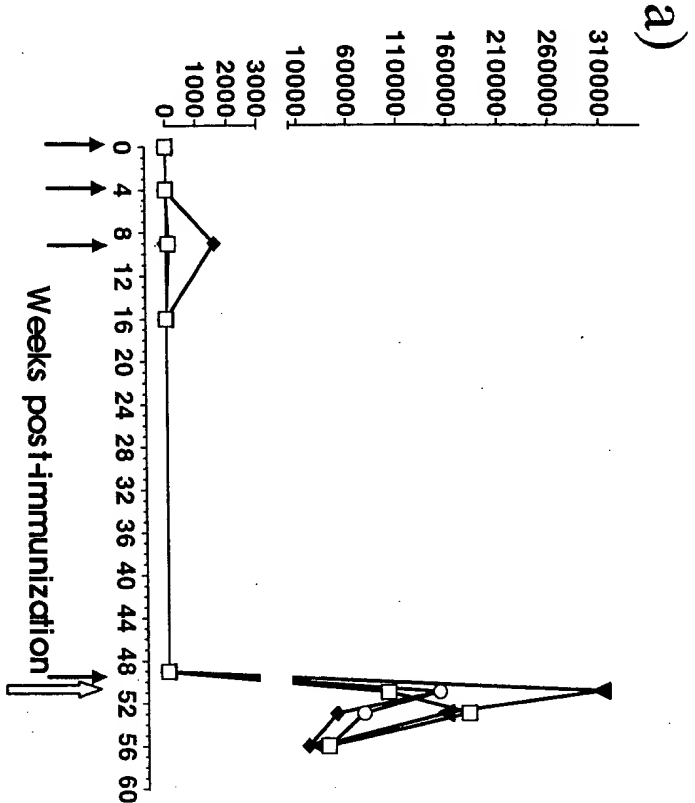


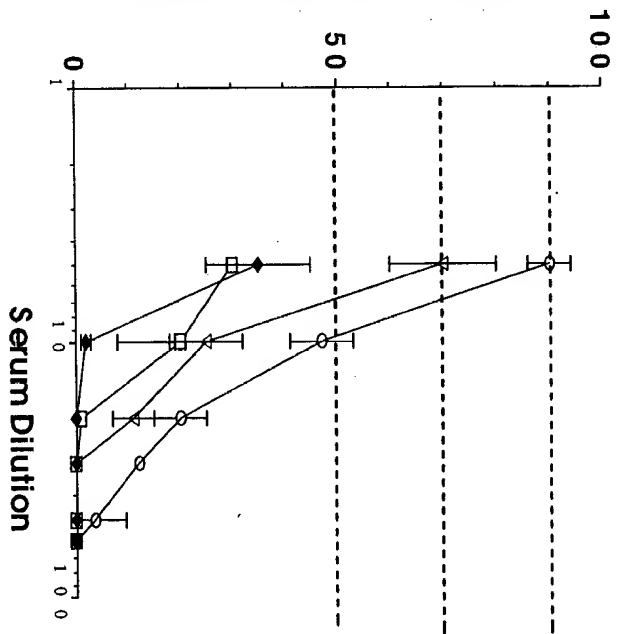
Fig 1

Fig 2

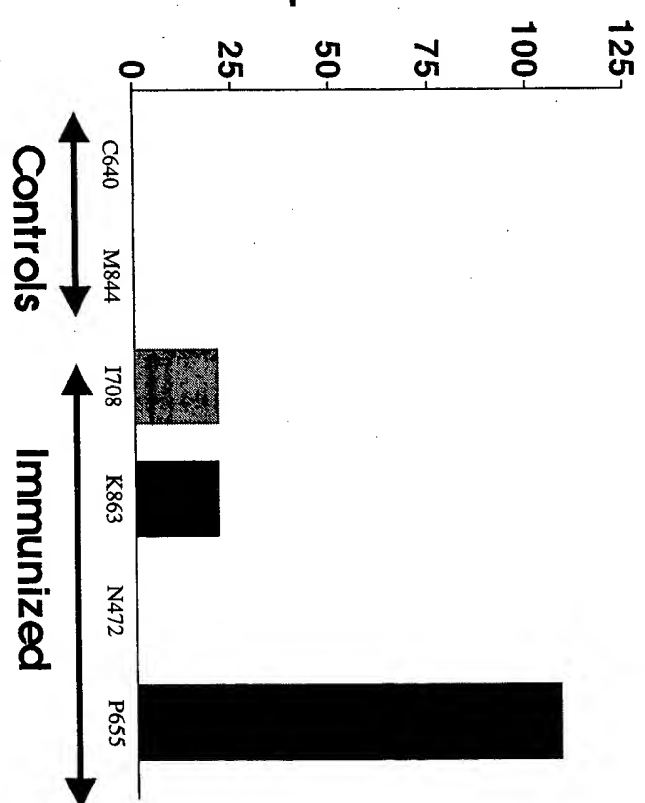
End-point ELISA antibody titers



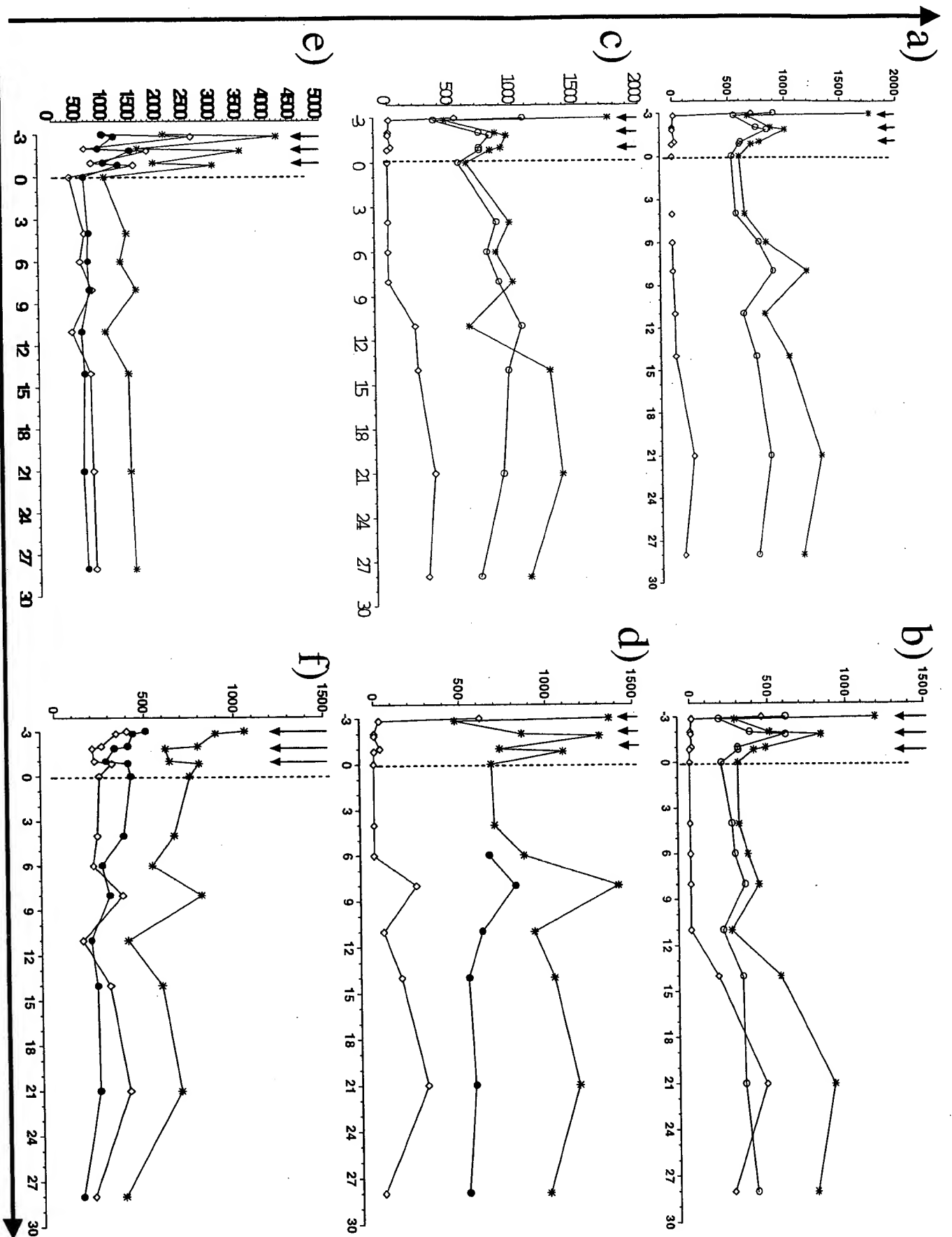
Percent inhibition of infection



SFC per 10⁶ PBMCs



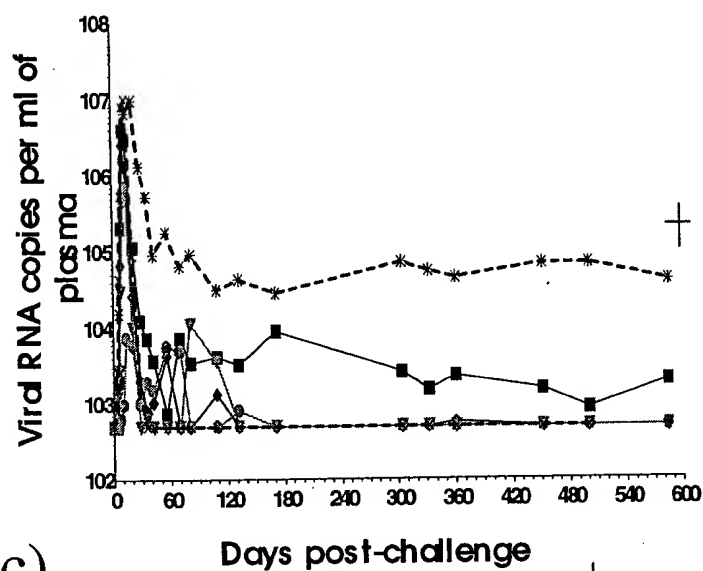
Number of T lymphocytes per ml of blood



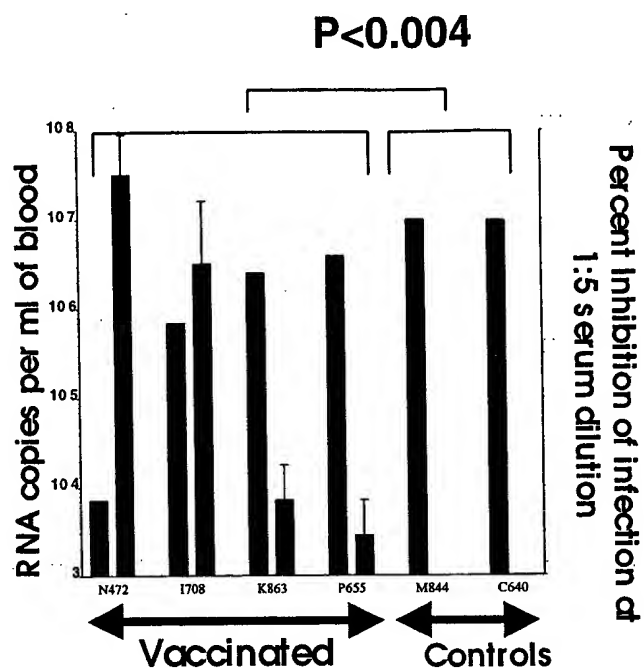
Days post-challenge

Fig 3

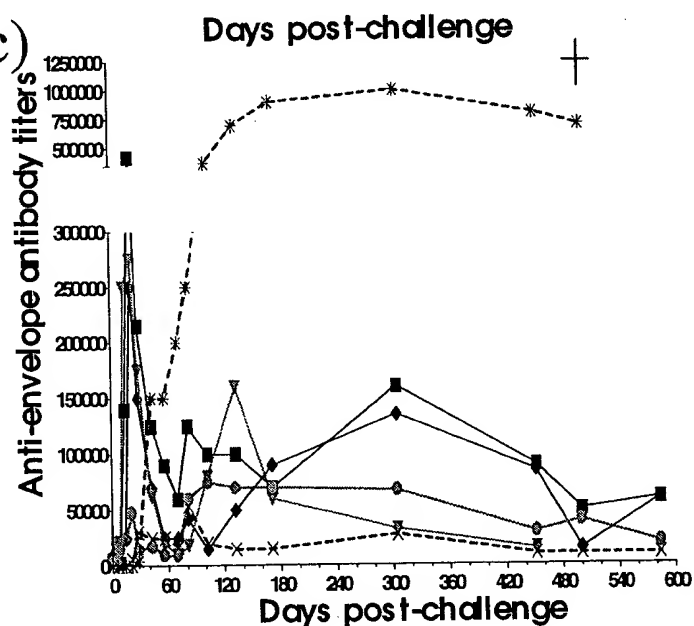
a)



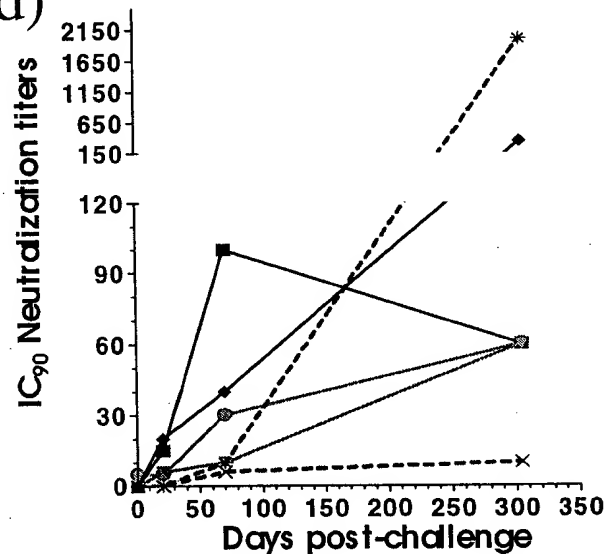
b)



c)



d)



e)

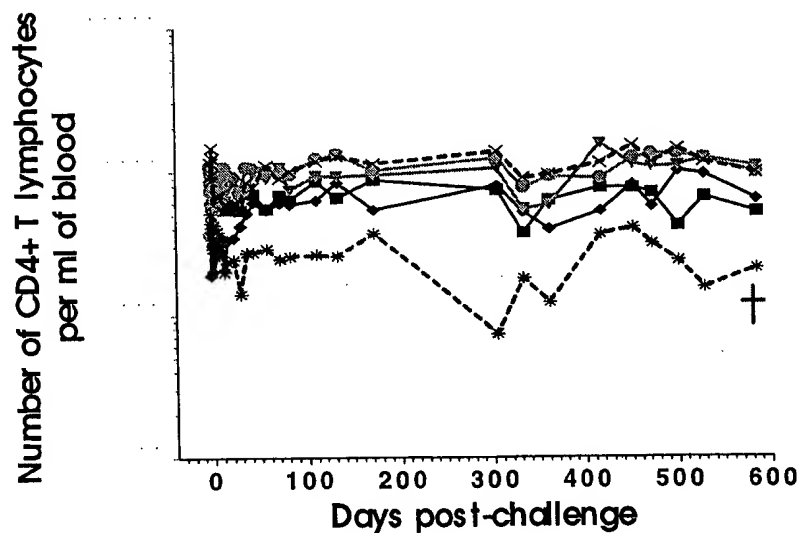


Fig 4

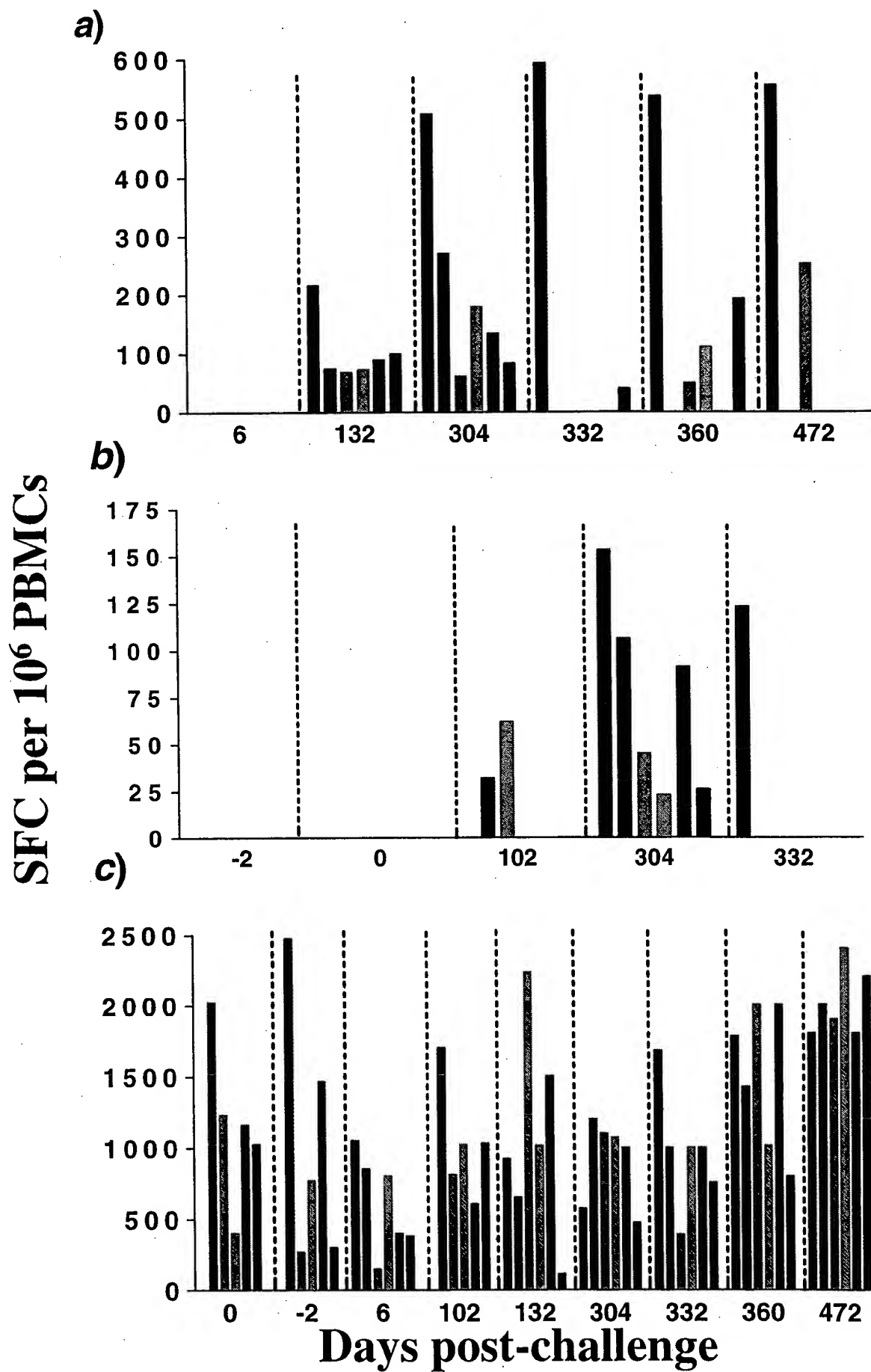


Fig 5